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MHC Class I–Restricted CTL Responses to Exogenous Antigens

Minireview

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Introduction

Different processing pathways operate for major histocompatibility complex (MHC)-restricted peptide presentation of exogenous and endogenous antigens (Townsend and Bodmer, 1989; Brodsky and Guagliardi, 1991; Yewdell and Bennink, 1992; Germain and Margulies, 1993). Exogenous antigens are internalized by antigen-presenting cells (APC) and degraded at an acid pH in vesicular intracellular compartments. Generated peptides are then loaded onto MHC class II (MHC-II) molecules and presented at the cell surface to $CD4^+$ helper T cells. In the cytoplasm, peptides are generated from endogenous antigens by degradation involving a multicatalytic proteinase complex, the proteasome. Cytosolic peptides are transported into the endoplasmic reticulum (ER) lumen by ATP-dependent transporters associated with antigen presentation (TAPs). In the ER lumen, peptides bind to nascent MHC class I (MHC-I) molecules in a chaperone-assisted assembly that generates stable trimeric MHC-I heavy chain–β_2-microglobulin–peptide complexes. These transport- and presentation-competent complexes move to the cell surface for recognition and activation of $CD8^+$ cytotoxic T lymphocytes (CTL). This clear-cut dichotomy between an exogenous processing pathway for MHC-II-restricted T cell response and an endogenous pathway for MHC-I-restricted T cell response is supported by extensive experimental data. However, recent data show that the injection of various types of exogenous antigens efficiently prime MHC-I-restricted CTL responses. Here, we briefly summarize these findings and discuss potential uptake mechanisms and processing pathways for exogenous antigens. Although little is yet known about alternative MHC-I processing pathways in APC, evidence for phagocytic and nonphagocytic processing pathways is emerging.

Exogenous Antigens That Prime MHC-I-Restricted CTL

Although injection of soluble proteins does not generate CTL responses in general, a growing number of modified exogenous protein antigens prime MHC-I-restricted CTL responses (see references in Table 1). Virus-infected cells, cells expressing minor histocompatibility antigens, and tumor cells can cross-prime MHC-I-restricted antigen-specific CTL, i.e., the transfer of antigen-bearing donor A cells generates B-restricted CTL reactivity in F1 (A × B) hosts. Although the mode of transfer of antigenic material from the transplanted, immunogenic cells to host-derived APC during cross-priming has not been elucidated, recent evidence for the shedding of antigenic vesicles (Zhou et al., 1992a; Raposo et al., 1996) and for the release of immunogenic, apoptotic blebs (Casciola-Rosen et al., 1996) provides interesting possibilities for antigen transfer to APC. Huang et al. (1996b) recently studied cross-priming using influenza virus nucleoprotein–transfected $H\text{-}2^d$ tumor cells given to irradiated $H\text{-}2^{dxb}$ mice rescued with bone marrow from $TAP^{-/-}$ $H\text{-}2^b$ mice. These mice developed a normal T cell compartment (on the $TAP^{+/+}$ thymic epithelial cells), which, however, could not respond to the nucleoprotein-transfected tumor cells, demonstrating a TAP dependency of the cross-priming effect. Whether the TAP dependency reflected processing of the antigen in the classical cytosolic class I pathway or a reduced amount of class I in an alternative processing compartment was not determined.

Injection of inactivated, noninfectious virus particles primes CTL responses, although higher doses than those used with the respective infectious, replicating virus are required. Association of exogenous antigens with some adjuvants, e.g., immune-stimulating complexes (ISCOMs), liposomes, squalene, or saponin, enhances their immunogenicity for CTL precursors. CTL responses are efficiently primed by the injection of protein antigens complexed with heat shock proteins (hsp). CTL priming by the injection of large, particulate antigens such as recombinant bacteria, crude cell lysates, denatured aggregates, and antigen-coupled beads, has been described. Immunogenicity of exogenous proteins for CTL is also strikingly increased by denaturation of protein antigens with anionic detergents. Some covalent modifications of proteins (lipid conjugation, cationization) convert them into potent inducers of CTL immunity. Multimeric virus-like particles assembled from surface or core viral proteins are surprisingly effective in stimulating MHC-I-restricted CTL when injected at low doses without adjuvants. Synthetic peptides, corresponding to immunodominant epitopes, generate strong CTL responses in vivo when given in isolation, mixed with adjuvants, or covalently or noncovalently associated with lipid carriers.

An important issue is the subcellular handling of these modified antigens after internalization into APC. Are all of these eventually transferred to the cytosolic compartment for conventional MHC-I processing, or does processing occur in alternative compartments? In eukaryotic cells, there is proteolytic activity in the cytosol, on the cell surface (e.g., ectopeptidases CD10, CD13, and CD26), and in vesicular compartments like the ER lumen, endosomes, and lysosomes (Dice and Terlecky, 1994; Authier et al., 1994; Ciechanover and Schwartz, 1994; Bonifacino and Klausner, 1994). Peptide processing for MHC-II presentation has been shown to occur at the cell surface; in a special lysosome-like compartment (MIIC or CIIV) containing newly synthesized MHC-II molecules; in early endosomes recruiting MHC-II molecules

Table 1. Exogenous Antigen Preparations That Prime MHC-I-Restricted T Cell Responses In Vivo

Antigen Formulations	Antigen System	References
Cross-priming	Minor H antigen Viral antigen (SV40 T-Ag) Tumor-associated antigens	Bevan, 1976a, 1976b; Knowles et al., 1979 Huang et al., 1994, 1996a, 1996b
Inactivated virus	Cytomegalovirus Sendai virus	Reddehase et al., 1984; Liu et al., 1995
ISCOM-associated	HIV gp120 Ovalbumin Measles virus	Takahashi et al., 1990; Heeg et al., 1991 van Binnendijk et al., 1992
Liposome-associated	Various protein antigens	Collins et al., 1992; Huang et al., 1992 Lopes and Chain, 1992; Miller et al., 1992 Nair et al., 1992, 1992b; Reddy et al., 1992 Zhou et al., 1992a, 1992b; Chen et al., 1993
Squalene	HIV gp120	Raychaudhuri et al., 1992
Saponin	HIV gp120 Ovalbumin	Wu et al., 1992, 1994 Newman et al., 1992
hsp-complexed	Tumor-associated antigen	Li and Srivastava, 1993; Srivastava et al., 1994; Srivastava, 1993; Undono et al., 1994 Undono and Srivastava, 1993, 1994
(Recombinant) bacteria	Various protein antigens	Aggarwal et al., 1990; Kaleab et al., 1990 Aldovini and Young, 1991; Stover et al., 1991, 1993; Schafer et al., 1992; Denkers et al., 1993; Pfeifer et al., 1993a, 1993b; Yasutomi et al., 1993a; Harding and Song, 1994; Szalay et al., 1994
Parasites		da Conceicao Silva et al., 1994 Denkers et al., 1993
Denatured aggregates	Surface antigen of HBV	Schirmbeck et al., 1995a
Antigen-coupled beads	Ovalbumin	Kovacsovics-Bankowski et al., 1993; Rock et al., 1993; Norbury et al., 1995
Denatured protein antigens	Ovalbumin HIV gp160 LCMV proteins Influenza virus proteins SV40 T-antigen Malaria CS protein Surface protein of HBV Pseudomonas exotoxin	Staerz et al., 1987; Schirmbeck et al., 1994a Doe et al., 1994; Schirmbeck et al., 1995b Bachmann et al., 1994; Weidt et al., 1994 Yamada et al., 1985; Wraith et al., 1987; Wraith and Vessey, 1986; Tevethia et al., 1980; Schirmbeck et al., 1992, 1993; Suss and Pink, 1992 Schirmbeck et al., 1994c; Ulmer et al., 1994
Lipid conjugation	Peptide vaccines	Deres et al., 1989; Schild et al., 1991a, 1991b Martinon et al., 1992; Nardelli and Tam, 1993; Vitiello et al., 1995
Multimeric protein particles	Yeast Ty particles HIV gag particles Surface particles of HBV	Harris et al., 1992; Layton et al., 1993 Schirmbeck et al., 1995b; Griffiths et al., 1993; Schirmbeck et al., 1994b, 1994d
Synthetic peptides with or without adjuvants		Alchele et al., 1990; Feltkamp et al., 1993 Deres et al., 1989; Lipford et al., 1994 Yasutomi et al., 1993b; Shirai et al., 1994 Zhou et al., 1992

by recycling from the cell surface; and in other subcellular organelles belonging to the endocytic system (Xu and Pierce, 1995).

Uptake of Exogenous Antigen by APC

To initiate a CTL response, MHC-bound peptides have to be presented to naive T cells by professional APC that express high levels of MHC-I molecules, adhesion receptors, and potent costimulator activity. APC internalize antigen locally and move to lymphoid tissues in two functionally distinct phases: uptake/transportation and T cell activation regulated by inflammatory stimuli such as TNFα, IL-1, and LPS (Steinman and Swanson, 1995; Lanzavecchia, 1996). The major APC for the generation of CTL responses are macrophages and dendritic cells (DC) (Young and Steinman, 1990; Debrick et al., 1991; Rock et al., 1993; Elbe et al., 1994; Harding and Song, 1994; Böhm et al., 1995). Macrophages ingest large particles by phagocytosis, involving cytoskeletal elements such as tubulin and actin for the formation of highly proteolytic phagosomes (Allen and Aderem, 1996). Macrophages can also collaborate with DC by degradation and transfer of smaller antigenic particles to these (Nair et al., 1995; Gong et al., 1994).

Table 2. Processing of Exogenous Antigen for MHC-I Expression In Vitro

Cellular Handling	Processing	Characteristics
Uptake and leakage to cytosol	Cytosolic MHC-I pathway[a]	Slow and inefficient. Require high Ag concentrations. Depend on proteasomes, TAP transporters, chaperones, and Golgi transport.
Uptake, digestion, and secretion	At membrane by "regurgitation"	Slow and inefficient. Require high Ag concentrations.
Uptake in a vesicular compartment	In an "endosomal-like" compartment	BFA resistant, TAP independent, and sensitive to endosomal inhibitors.
Peptide binding to "empty" MHC-I	Not required	Sensitizes target cells.

[a] Cytosolic protein can also enter an "endosomal-like" pathway through hsp-mediated uptake (Schirmbeck and Reimann, 1994).

Nonphagocytic uptake of antigen into APC can be either receptor operated or in the fluid phase by pinocytosis, macropinocytosis, and phagocytosis (Steinman, 1991; Reis e. Sousa et al., 1993; Sallusto et al., 1995). Macropinocytosis in DC is constitutive and highly efficient and leads to the concentration of internalized material into intracellular vesicular processing compartments (Lanzavecchia, 1996). A receptor-operated uptake can either depend on mannose, scavenger-type receptors (such as DEC-205 in the mouse) or other, less defined receptors (Pearson, 1996; Manca et al., 1991; Ezekowitz et al., 1990; Stahl, 1992; Jiang et al., 1995).

Consequently, a number of different uptake pathways operate in APC with the capacity to handle antigen differently: complete degradation, partial degradation and secretion, partial degradation and leakage to the cytosol (see below), or uptake into vesicular processing compartments. Different uptake mechanisms might also target the antigen to different processing compartments, such as the cell membrane, early endosomes, lysosomal-like vesicles, or the cytosol. Different peptide repertoires for MHC binding and presentation might potentially be generated in these distinct compartments.

Processing of Exogenous Antigen for MHC-I-Restricted Peptide Presentation

Processing has been studied at different levels, including target cell recognition and the capacity of cells to generate CTL in in vitro cultures. Target cell recognition is a comparatively simple event requiring only short-term expression of a few presentation-competent trimeric complexes to trigger CTL lysis (Christinck et al., 1991). Both normal and TAP mutant cells and cell lines have been used. Pathways have been defined further by cellular inhibitors selective for Golgi transport (brefeldin A [BFA]) or proteasomal activity (lactacystin and peptide aldehydes) and those that target endosomal functions such as vesicular pH, proteolysis, and transport (Table 2).

Exogenous antigens such as recombinant bacteria, bead-coupled or aggregated proteins, or antigens incorporated into liposomes or ISCOM particles are internalized into macrophages by phagocytosis and can enter different processing pathways for MHC-I-restricted presentation of peptides (Harding and Song, 1994; Kovacsovics-Bankowski et al., 1993; Reis e. Sousa et al., 1993; Pfeifer et al., 1993b; Falo et al., 1995; Bachmann et al., 1995; Rock, 1996). Some antigens can escape from phagosomes into the cytosolic compartment, where they presumably enter the endogenous processing pathway as judged by the inhibitory effect of BFA or proteasomal inhibitors (or both) (Takahashi et al., 1990; van Binnendijk et al., 1992; Aggarwal et al., 1990; Aldovini and Young, 1991; Stover et al., 1991, 1993; Schafer et al., 1992; Rock, 1996). Little is presently known about this "leakage" from phagosomes to the cytosol: whether it is an energy-dependent process regulated by specific transporters, whether it depends on unspecified damage to the vesicular membrane, or whether it represents an important in vivo event. The phagosome to cytosol transfer process has been demonstrated with internalized toxin, which lacks the capacity to penetrate membranes but still can enter the cytosol to act on ribosomes (Reis e. Sousa and Germain, 1995; Norbury et al., 1995; Kovacsovics-Bankowski and Rock, 1995), with fluoresceinated dextran and ISCOM-associated protein antigen, and in different intracellular microbial systems (Norbury et al., 1995; Andrews, 1994; Morein et al., 1994). There is also evidence for a bidirectional transfer of peptides and proteins between lysosomes and the cytosol, including fusion between autophagosomes and lysosomes or hsp-73-mediated antigen transfer (Isenman and Dice, 1989; Dice, 1992; Mortimore and Kadowaki, 1994; Schirmbeck and Reimann, 1994).

Secreted peptides from partially digested antigens in phagosomes can bind membrane MHC-I by "regurgitation." These peptides can also bind to MHC-I on "bystander" cells in the immediate vicinity of the processing cells (Pfeifer et al., 1993a; Rock, 1996). This has been found with ovalbumin (either present as bacterial fusion protein or bound to beads) in some studies, but not in others (Pfeifer et al., 1993b; Reis e. Sousa and Germain, 1995; Rock, 1996). It thus remains to be determined whether partially digested antigens from phagocytes can actually be processed for MHC-I presentation by the regurgitation process, or whether it is an in vitro phenomenon that readily happens with fixed cells. A more likely alternative may be transfer of partially digested antigen into DC through any of the multiple mechanisms these cells can use for internalization of antigen.

Phagocytic processing of exogenous antigens, in both professional and nonprofessional phagocytes, is more efficient if the antigen is either coupled or mixed with beads that by themselves have the capacity to activate phagosomes (Huang et al., 1994; Kovacsovics-Bankowski et al., 1993; Rock et al., 1993; De Bruijn et al., 1995). A closer analysis of phagocytic processing by Reis e. Sousa and Germain (1995) and Schirmbeck et al. (1995c), comparing peptide- and bead-associated

antigens, characterized it as inefficient and related to phagocytic overload ("cellular indigestion"). Phagocytic processing of many antigens is thus slow, requires high antigen concentrations, and is potentially risky as it might involve sensitization of innocent bystander cells.

Nonphagocytic processing of exogenous antigens for MHC-I-restricted presentation of peptides has been demonstrated with inactivated virus particles, surface or core virus-like particles, and glycopeptides (Rock, 1996; Abdel Motal et al., 1993, 1996; Schirmbeck and Reimann, 1994; Schirmbeck et al., 1995a, 1995c; Zhou et al., 1995). This pathway is rapid and requires low doses of antigen to elicit a response. It is resistant to BFA, operates in mutant cells that express no TAP peptide transporter activity, and is also blocked by lipophilic amines that raise endosomal pH, affect uptake mechanisms, and inhibit vesicular trafficking. These effects indicate that processing of exogenous antigens for MHC-I expression relies on mechanisms similar to those operating in the MHC-II pathway. There are also additional similarities, as both MHC-I and MHC-II molecules recycle between an intracellular compartment and the cell surface (Yewdell and Bennink, 1992; Wraith and Vessey, 1986; Wraith et al., 1987; Reid and Watts, 1990). In vivo data from a transgenic mouse model suggest a role for recycling of MHC-I in the priming event of CTL responses. Two lines of transgenic mice were constructed (on a DBA background), one of which expressed the class I molecule D^b in its normal, transmembranous, and recycling form and the other of which expressed a glycosylphosphatidylinositol (GPI)-linked form of D^b that could not recycle, or at least had a severely reduced capacity to recycle. Only mice that expressed the normal, recycling D^b molecules, and not mice that expressed the GPI-linked, nonrecycling form of D^b molecules, could generate a CTL response against an immunodominant epitope in the influenza A virus nucleoprotein after virus infection (Abdel Motal et al., 1995b).

An endosomal processing compartment would need an input of MHC-I molecules that can bind processed peptides. MHC-I molecules could be derived by recycling from the cell surface, from the formation of membrane vesicles containing correctly positioned MHC-I molecules, or by direct transport from the ER compartment. Recycling of class I molecules between the cell membrane and an endosomal compartment has been demonstrated in both T cells and macrophages and shown to exceed the de novo synthesis pathway in terms of membrane expression (Yewdell and Bennink, 1992; Reid and Watts, 1990; Dasgupta et al., 1988). The cytoplasmic tail of MHC-I molecules contains phosphorylation sites that might regulate recycling to allow a selective accumulation of presentation-competent complexes, although this is hypothetical (Vega and Strominger, 1989). Phagocytic vesicles, derived from the plasma membrane, might contain peptide-receptive class I molecules, or newly synthesized class I molecules might be directly transported to these with the help of chaperone proteins (De Bruijn et al., 1995). Eventually, these class I molecules might be transferred to an endocytic compartment by vesicular transport. The invariant chain, used for trafficking of class II molecules, has been shown to direct a subset of MHC-I molecules directly to the endocytic compartment, even if the peptide presentation capacity of such chaperoned MHC-I molecules has not been determined (Sugita and Brenner, 1995). Glycosylated class I–binding peptides, which bind to cells expressing the corresponding restriction element with high specificity and are easily detected with specific anti-carbohydrate monoclonal antibodies by fluorescence-activated cell sorting, are readily recycled to the cell membrane after internalization. This process is blocked by endosomal inhibitors (Abdel Motal et al., 1993, 1995a). It is not clear whether peptides are recycling on the same class I molecules that are used for internalization or whether an active peptide exchange occurs in the endosomal compartment. The internalization process is, however, important for the formation of presentation-competent trimeric class I complexes (see below). The low pH in endosomes may favor peptide exchange and the formation of presentation-competent complexes, as well as influencing the expressed peptide repertoire. pH is known to influence the spectrum of peptides that bind class I molecules (Stryhn et al., 1996).

Alternative configurations of MHC-I molecules are expressed on the cell surface, such as stable trimeric complexes (including β_2-microglobulin light chains and antigenic peptides) or "empty" heavy chains, which are not loaded with peptides and not stably associated with β_2-microglobulin (Lie et al., 1990, 1991; Matko et al., 1994; Carreno and Hansen, 1994; Smith et al., 1992a, 1992b; 1993; Allen et al., 1986; Jackson and Peterson, 1993; Jackson et al., 1994). "Empty" MHC-I molecules, stably expressed at 37°C on the surface of normal and, in particular, TAP mutant cells, may arise from peptide or β_2-microglobulin dissociation of assembled trimeric complexes (or both) (Bernabeau et al., 1984; Rock et al., 1990, 1991; Schuhmacher et al., 1990; Vitiello et al., 1990; Kozlowski et al., 1991). Alternatively, empty MHC-I heavy chains may be transported from the ER to the cell surface as free heavy chains (Allen et al., 1986). Chaperone molecules may be involved in the expression of empty MHC-I molecules at the cell surface and in their recycling through endosomal-like compartments. A recent report described the heterodimerization of empty L^d molecules with T3-encoded Qa-1 molecules on the cell surface (Wolf and Cook, 1995). However, surface-expressed empty MHC-I molecules at 37°C do not directly bind exogenous peptides, i.e., empty MHC-I molecules are not converted into presentation-competent trimeric complexes by binding exogenous peptides (Lie et al., 1990, 1991; Smith et al., 1992a, 1992b, 1993). These empty class I molecules may have to be internalized before they can be loaded with antigenic peptides from endocytosed, exogenous antigens and associate with β_2-microglobulin to generate presentation-competent trimeric complexes. Exogenous 22 nm hepatitis B surface antigen (HBsAg) particles are taken up by many cells and processed in a novel peptide transporter–independent, endosomal, or lysosomal pathway for class I (L^d)-restricted epitope presentation. Empty L^d molecules defined by specific monoclonal antibodies, which are derived from the cell surface, are involved in presenting antigenic peptides from endocytosed HBsAg

particles (Schirmbeck and Reimann, 1996). Another species of empty MHC-I molecules can be artificially induced (by low temperature on TAP mutant cells) and loaded with exogenous peptides (Schuhmacher et al., 1990; Ljunggren et al., 1990; Machold et al., 1995). However, these empty MHC-I molecules are thermolabile and hence not equivalent to empty forms expressed under physiological conditions.

Synthetic peptides that have the correct MHC-I binding motif and correspond to known CTL epitopes (usually 8–12 amino acids long) do not require any processing and generate CTL responses in vivo (Alchele et al., 1990; Feltkamp et al., 1993; Yasutomi et al., 1993b; Zhou et al., 1992). As discussed above, these probably bind to empty MHC-I molecules in an intracellular processing compartment to form presentation-competent complexes. The in vivo responses to short, synthetic peptides can be independent of $CD4^+$ helper T cells, as shown with both CD4 knockout mice and anti-CD4-treated mice (Zhou et al., 1992b; Fayolle et al., 1996).

Studies in TAP knockout mice give further support for an alternative MHC-I processing pathway. Although these mice have severely reduced MHC-I membrane levels and do not generate virus-specific CTL responses, they can respond to other antigens such as alloantigens, tumors, and short peptides representing immunodominant epitopes (Ljunggren et al., 1995a, 1995b; van Kaer et al., 1992; Sandberg et al., 1996). The inability to generate virus-specific CTL may reflect a reduced input of MHC-I molecules into the processing compartment, since class I heavy chains mostly remain in the ER in these mice as a consequence of the TAP deficiency. However, other antigens, to which these mice can respond, might internalize more readily into the processing compartment and thus efficiently capture those few MHC-I molecules that have escaped from the ER compartment.

Conclusions

A number of different modified exogenous protein antigens can efficiently prime CTL responses. The biological significance of this for priming of normal CTL responses is unknown. Processing of exogenous antigen for MHC-I presentation seems an attractive possibility to bypass the necessity that all foreign antigens have to be translated within the cytosol of professional APC in order to prime CTL responses. Many microbes have a select host cell specificity that does not include APC. They kill their host cells or severely disturb the sophisticated cellular functions that are required in APC (Norkin, 1995; Haywood, 1994). Another scenario for a virus-specific CTL response is thus that infected cells disintegrate (possibly as apoptotic bodies) and exogenous antigen from them is transferred to APC for further processing in different cellular compartments.

There are many unresolved issues regarding processing of exogenous antigens for MHC-I presentation and the priming of CTL responses. First, which are the most important uptake mechanisms? Second, is there a special (MHC-II-like) compartment for "endosomal" processing, or do all APC "leak" antigen into the cytosol and thereby into the classical MHC-I processing pathway? Third, in a proposed endosomal-like compartment, which are the proteolytic enzymes involved in processing? Do they generate a spectrum of MHC-I-bound peptides distinct from those generated in the cytosolic pathway? Finally, how do MHC-I molecules gain access to the endosomal-like compartment, and how are trimeric peptide MHC-I–β_2-microglobulin complexes transported to the cell surface and their membrane expression regulated?

If exogenous antigens are instrumental in priming normal CTL responses, processing in noncytosolic, vesicular compartments, distinct from the endogenous classical class I processing pathway, might be important. If so, it may be relevant to reevaluate the endogenous/MHC-I and exogenous/MHC-II processing dogma and instead classify pathways in functional terms. In CTL activation, induction (by exogenous antigen) and target cell recognition (by endogenous antigen) by class I–restricted peptide presentation would result from different processing pathways. This is an important issue in terms of vaccine development, as these should primarily be expressed in the most optimal processing compartment. If exogenous antigens are of major importance in generating CTL responses, vaccines based on live, replicating vectors (and nucleic acids) induce their protective effect in a nonviable form. In this transfer process, from the infected host cell to the professional APC cell, there might be a lesson to be learned in terms of formulations for direct uptake into APC cells. The potent clinical effect of the first human recombinant vaccine licensed for clinical use, which was based on self-assembly of the hepatitis B virus small surface antigen into 22 nm subviral particles, might be a "lesson of nature" in this context (Schirmbeck et al., 1996).

References

Abdel Motal, U.M., Zhou, X., Joki, A., Siddiqi, A.R., Srinivasa, B.R., Stenvall, K., Dahmén, J., and Jondal, M. (1993). Eur. J. Immunol. *23*, 3224–3229.

Abdel Motal, U.M., Berg, L., Bengtsson, M., Dahmén, J., Kihlberg, J., Magnusson, G., Nilsson, U., and Jondal, M. (1995a). J. Immunol. Meth. *188*, 21–31.

Abdel Motal, U.M., Sentman, C.L., Zhou, X., Robinson, P.J., Dahmén, J., and Jondal, M. (1995b). Eur. J. Immunol. *25*, 1121–1124.

Abdel Motal, U.M., Berg, L., Rosén, A., Bengtsson, M., Thorpe, C.J., Kihlberg, J., Dahmén, J., Magnusson, G., Karlsson, K.-A., and Jondal, M. (1996). Eur. J. Immunol. *26*, 544–551.

Aggarwal, A., Kumar, S., Jaffe, R., Hone, D., Gross, M., and Sadoff, J. (1990). J. Exp. Med. *172*, 1083–1090.

Alchele, P., Hengartner, H., Zinkernagel, R.M., and Schulz, M. (1990). J. Exp. Med. *171*, 1815–1826.

Aldovini, A., and Young, R.A. (1991). Nature *351*, 479–482.

Allen, H., Fraser, J., Flyer, D., Calvin, S., and Flavell, R.H. (1986). Proc. Natl. Acad. Sci. USA *83*, 7447–7451.

Allen L.-E.H., and Aderem, A. (1996). Curr. Opin. Immunol. *8*, 36–40.

Andrews, N.W. (1994). Braz. J. Med. Biol. Res. *27*, 471–475.

Authier, F., Posner, B.I., and Bergeron, J.J.M. (1994). In Cellular Proteolytic Systems, A.J. Ciechanover and A.L. Schwartz, eds. (New York: Wiley–Liss), pp. 89–113.

Bachmann, M.F., Kündig, T.M., Freer, G., Li, Y., Kang, C.Y., Bishop, D.H., Hengartner, H., and Zinkernagel, R.M. (1994). Eur. J. Immunol. *24*, 2128–2236.

Bachmann, M.F., Oxenius, A., Pircher, H., Hengartner, H., Ashton-Richardt, P.A., Tonegawa, S., and Zinkernagel, R.M. (1995). Eur. J. Immunol. *25*, 1739–1743.

Bernabeau, C., van de Rijin, M., Lerch, P.G., and Terhorst, C. (1984). Nature *308*, 642–645.

Bevan, M.J. (1976a). J. Exp. Med. *143*, 1283–1288.

Bevan, M.J. (1976b). J. Immunol. *117*, 2233–2238.

Böhm, W., Schirmbeck, R., Elbe, A., Melber, K., Diminsky, D., Kraal, G., Van Rooijen, N., Barenholz, Y., and Reimann, J. (1995). J. Immunol. *155*, 3313–3321.

Bonifacino, J.S., and Klausner, R.D. (1994). In Cellular Proteolytic Systems, A.J. Ciechanover and A.L. Schwartz, eds. (New York: Wiley–Liss), pp. 137–161.

Brodsky, F.M., and Guagliardi, L.E. (1991). Annu. Rev. Immunol. *9*, 707–744.

Carreno, B.M., and Hansen, T.H. (1994). Eur. J. Immunol. *24*, 1285–1292.

Casciola-Rosen, L., Rosen, A., Petri, M., and Schissel, M. (1996). Proc. Natl. Acad. Sci. USA *93*, 1624–1629.

Chen, W.-F., Carbone, F.R., and McCluskey, J. (1993). J. Immunol. Meth. *160*, 49–57.

Christinck, E.R., Luscher, M.A., Barber, B.H., and Williams, D.B. (1991). Nature *352*, 67–70.

Ciechanover, A.J., and Schwartz, A.L. (1994). In Cellular Proteolytic Systems, A.J. Ciechanover and A.L. Schwartz, eds. (New York: Wiley–Liss), pp. 3–21.

Collins, D.S., Findlay, K., and Harding, C.V. (1992). J. Immunol. *148*, 3336–3341.

da Conceicao Silva, F., Perlaza, B.L., Louis, J.A., and Romero, P. (1994). Eur. J. Immunol. *24*, 2813–2817.

Dasgupta, J.D., Watkins, S., Slayter, H., and Yunis, E.J. (1988). J. Immunol. *141*, 2577–2580.

Debrick, J.E., Campbell, P.A., and Staerz, U.D. (1991). J. Immunol. *14*, 2846–2851.

De Bruijn, M.L.H., Jackson, M.R., and Peterson, P.A. (1995). Eur. J. Immunol. *25*, 1274–1285.

Denkers, E.Y., Gazzinelli, R.T., Hieny, S., Caspar, P., and Sher, A. (1993). J. Immunol. *150*, 517–526.

Deres, K., Schild, H., Wiesmüller, K.-H., Jung, G., and Rammensee, H.G. (1989). Nature *342*, 561–564.

Dice, J.F. (1992). Ann. NY Acad. Sci. *674*, 58–64.

Dice, J.F., and Terlecky, S.R. (1994). In Cellular Proteolytic Systems, A.J. Ciechanover and A.L. Schwartz, eds. (New York: Wiley–Liss), pp. 55–64.

Doe, B., Steimer, K.S., and Walker, C.M. (1994). Eur. J. Immunol. *24*, 2369–2376.

Elbe, A., Schleischitz, S., Strunk, D., and Stingl, G. (1994). J. Immunol. *153*, 2878–2889.

Ezekowitz, R.A., Sastry, K., Bailly, P., and Warner, A. (1990). J. Exp. Med. *172*, 1785–1794.

Falo, L.D., Jr., Kovacsovics Bankowski, M., Thompson, K., and Rock, K.L. (1995). Nature Med. *1*, 649–653.

Fayolle, C., Abdel-Motal, U.M., Berg, L., Deriaud, E., Jondal, M., and Leclerc, C. (1996). Immunology *89*, 41–45.

Feltkamp, M.C., Smits, H.L., Vierboom, M.P., Minnaar, R.P., de Jongh, B.M., Drijfhout, J.W., ter Schegget, J., Melief, C.J., and Kast, W.M. (1993). Eur. J. Immunol. *23*, 2242–2249.

Germain, R.N., and Margulies, D.H. (1993). Annu. Rev. Immunol. *11*, 403–450.

Gong, J.L., McCarthy, K.M., Rogers, R.A., and Schnasberger, S.E. (1994). Immunology *81*, 343–351.

Griffiths, J.C., Harris, S.J., Layton, G.T., Berrie, E.L., French, T.J., Burns, N.R., Adams, S.E., and Kingsman, A.J. (1993). J. Virol. *67*, 3191–3198.

Harding, C.V., and Song, R. (1994). J. Immunol. *153*, 4925–4933.

Harris, S.J., Gearing, A.J., Layton, G.T., Adams, S.E., and Kingsman, A.J. (1992). Immunology *77*, 315–321.

Haywood, A.M. (1994). J. Virol. *68*, 1–5.

Heeg, K., Kuon, W., and Wagner, H. (1991). Eur. J. Immunol. *21*, 1523–1527.

Huang, A.Y., Golumbek, P., Ahmadzadeh, M., Jaffee, E., Pardoll, D., and Levitsky, H. (1994). Science *264*, 961–965.

Huang, A.Y., Bruce, A.T., Pardoll, D.M., and Levitsky, H.I. (1996a). J. Exp. Med. *183*, 769–776.

Huang, A.Y.C., Bruce, A.T., Pardoll, D.M., and Levitsky, H.I. (1996b). Immunity *4*, 349–355.

Huang, L., Reddy, R., Nair, S.K., Zhou, F., and Rouse, B.T. (1992). Res. Immunol. *143*, 192–196.

Isenman, L.D., and Dice, J.F. (1989). J. Biol. Chem. *264*, 21591–21596.

Jackson, M.R., and Peterson, P.A. (1993). Annu. Rev. Cell Biol. *9*, 207–235.

Jackson, M.R., Cohen Doyle, M.F., Peterson, P.A., and Williams, D.B. (1994). Science *263*, 384–387.

Jiang, W., Swiggard, W.J., Heufler, C., Peng, M., Mirza, A., Steinman, R., and Nussenzweig, M.C. (1995). Nature *375*, 151–155.

Kaleab, B., Ottenhoff, T.H.M., Converse, P., Halapi, E., Tadesse, G., Rottenberg, M., and Kiessling, R. (1990). Eur. J. Immunol. *20*, 2651–2659.

Knowles, B.B., Koncar, M., Pfizenmaier, K., Solter, D., Aden, D.P., and Trinchieri, G. (1979). J. Immunol. *122*, 1798–1805.

Kovacsovics-Bankowski, M., and Rock, K.L. (1995). Science *267*, 243–246.

Kovacsovics-Bankowski, M., Clark, K., Benacerraf, B., and Rock, K.L. (1993). Proc. Natl. Acad. Sci. USA *90*, 4942–4946.

Kozlowski, S., Takeshita, T., Boehnke, W.-H., Takahashi, H., Boyd, L.F., Germain, R.N., Berzofsky, J.A., and Margulies, D.H. (1991). Nature *349*, 74–77.

Lanzavecchia, A. (1996). Curr. Opin. Immunol. *8*, 348–354.

Layton, G.T., Harris, S.J., Gearing, A.J., Hill Perkins, M., Cole, J.S., Griffiths, J.C., Burns, N.R., Kingsman, A.J., and Adams, S.E. (1993). J. Immunol. *151*, 1097–1107.

Li, Z., and Srivastava, P.K. (1993). EMBO J. *12*, 3143–3151.

Lie, W.-R., Myers, N.B., Gorka, J., Rubocki, R.J., Conolly, J.M., and Hansen, T.H. (1990). Nature *344*, 439–441.

Lie, W.-R., Myers, N.B., Conolly, J.M., Gorka, J., Lee, D.R., and Hansen, T.H. (1991). J. Exp. Med. *173*, 449–459.

Lipford, G.B., Wagner, H., and Heeg, K. (1994). Vaccine *12*, 73–80.

Liu, T., Zhou, X., Orvell, C., Lederer, E., Ljunggren, H.-G., and Jondal, M. (1995). J. Immunol. *154*, 3147–3155.

Ljunggren, H.-G., Stan, N.J., Öhlén, C., Neefjes, J.J., Höglund, P., Heemels, M.-T., Bastin, J., Schumacher, T., Townsend, A., Kärre, K., and Ploegh, H.L. (1990). Nature *346*, 476–480.

Ljunggren, H.-G., van Kaer, L., Sabatine, M.S., Auchincloss, H., Jr., Tonegawa, S., and Ploegh, H.L. (1995a). Int. Immunol. *7*, 975–984.

Ljunggren, H.-G., van Kaer, L., Ashton-Rickardt, P.G., Tonegawa, S., and Ploegh, H.L. (1995b). Eur. J. Immunol. *25*, 174–178.

Lopes, L.M., and Chain, B.M. (1992). Eur. J. Immunol. *22*, 287–290.

Machold, R.P., Andree, S., van Kaer, L., Ljunggren, H.-G., and Ploegh, H.L.J. (1995). J. Exp. Med. *181*, 1111–1122.

Manca, F., Fenglio, D., Li-Pira, G., Kunkl, A., and Celada, F. (1991). J. Exp. Med. *173*, 37–48.

Martinon, F., Gras-Masse, H., Boutillon, C., Chirat, F., Deprez, B., Guillet, J.-G., Gomard, E., Tartar, A., and Levy, J.-P. (1992). J. Immunol. *149*, 3416–3422.

Matko, J., Bushkin, Y., Wei, T., and Edidin, M. (1994). J. Immunol. *152*, 3353–3360.

Miller, M.D., Gould-Fogerite, S., Shen, L., Woods, R.M., Koenig, S., Mannino, R.J., and Letvin, N.L. (1992). J. Exp. Med. *176*, 1739–1744.

Morein, B., Villacrema-Eriksson, M., Åkerblom, L., Rönnberg, B., Lövgren K., and Sjölander, A. (1994). AIDS Res. Hum. Retroviruses *10* (Suppl. 2), 109–114.

Mortimore, G.E., and Kadowaki, M. (1994). In Cellular Proteolytic

Systems, A.J. Ciechanover and A.L. Schwartz, eds. (New York: Wiley–Liss), pp. 65–87.

Nair, S., Zhou, F., Reddy, R., Huang, L., and Rouse, B.T. (1992a). J. Exp. Med. *175*, 609–612.

Nair, S., Zhou, X., Huang, L., and Rouse, B.T. (1992b). J. Immunol. Meth. *152*, 237–243.

Nair, S., Buiting, A.M.J., Rouse, R.J., Van Rooijen, N., Huang, L., and Rouse, B.T. (1995). Int. Immunol. *4*, 679–688.

Nardelli, B., and Tam, J.P. (1993). Immunology *79*, 355–361.

Newman, M.J., Wu, J.Y., Gardner, B.H., Munroe, K.J., Leombruno, D., Recchia, J., Kensil, C.R., and Coughlin, R.T. (1992). J. Immunol. *148*, 2357–2362.

Norbury, C.C., Hewlett, L.J., Prescott, A.R., Shastri, N., and Watts, C. (1995). Immunity *3*, 783–791.

Norkin, L.C. (1995). Clin. Microbiol. Rev. *8*, 293–315.

Pearson, A.M. (1996). Curr. Opin. Immunol. *8*, 20–28.

Pfeifer, J.D., Wick, M.J., Harding, C.V., and Normark, S.J. (1993a). Infect. Agents Dis. *2*, 249–254.

Pfeifer, J.D., Wick, M.J., Roberts, R.L., Findlay, K., Normark, S.J., and Harding, C.V. (1993b). Nature *361*, 359–362.

Raposo, G., Nijman, H.W., Stoorvogel, W., Leijendekker, R., Harding, C.V., Melief, C.J., and Geuze, H.J. (1996). J. Exp. Med. *183*, 1161–1172.

Raychaudhuri, S., Tonks, M., Carbone, F., Ryskamp, T., Morrow, W.J., and Hanna, N. (1992). Proc. Natl. Acad. Sci. USA *89*, 8308–8312.

Reddehase, M.J., Keil, G.M., and Koszinowski, U.H. (1984). Eur. J. Immunol. *14*, 56–61.

Reddy, R., Zhou, F., Nair, S., Huang, L., and Rouse, B.T. (1992). J. Immunol. *148*, 1585–1589.

Reid, P.A., and Watts, C. (1990). Nature *346*, 655–657.

Reis e. Sousa, C., and Germain, E.R. (1995). J. Exp. Med. *182*, 841–851.

Reis e. Sousa, C., Stahl, P.D., and Austyn, J.M. (1993). J. Exp. Med. *178*, 509–519.

Rock, K.L. (1996). Immunol. Today *17*, 131–137.

Rock, K.L., Rothstein, L., Gamble, S., and Benacerraf, B. (1990). Proc. Natl. Acad. Sci. USA *87*, 7517–7521.

Rock, K.L., Gamble, S., Rothstein, L., Gramm, G., and Benacerraf, B. (1991). Cell *65*, 611–620.

Rock, K.L., Rothstein, L., Gamble, S., and Fleischacker, C. (1993). J. Immunol. *150*, 438–446.

Sallusto, F., Cella, M., Danieli, C., and Lanzavecchia, A. (1995). J. Exp. Med. *182*, 389–400.

Sandberg, J.K., Chambers, B.J., van Kaer, L., Kärre, K., and Ljunggren, H.-G. (1996). Eur. J. Immunol. *26*, 288–293.

Schafer, R., Portnoy, D.A., Brassell, S.A., and Paterson, Y. (1992). J. Immunol. *149*, 53–59.

Schild, H., Deres, K., Wiesmüller, K.-H., Jung, G., and Rammensee, H.G. (1991a). Eur. J. Immunol. *21*, 2649–2654.

Schild, H., Norda, M., Deres, K., Falk, K., Rötzschke, O., Wiesmüller, K.-H., Jung, G., and Rammensee, H.G. (1991b). J. Exp. Med. *174*, 1665–1668.

Schirmbeck, R., and Reimann, J. (1994). Eur. J. Immunol. *24*, 1478–1486.

Schirmbeck, R., and Reimann, J. (1996). Eur. J. Immunol., in press.

Schirmbeck, R., Zerrahn, J., Kuhröber, A., Kury, E., Deppert, W., and Reimann, J. (1992). Eur. J. Immunol. *22*, 759–766.

Schirmbeck, R., Zerrahn, J., Kuhröber, A., Deppert, W., and Reimann, J. (1993). Eur. J. Immunol. *23*, 1528–1534.

Schirmbeck, R., Böhm, W., and Reimann, J. (1994a). Eur. J. Immunol. *24*, 2068–2072.

Schirmbeck, R., Melber, K., Kuhröber, A., Janowicz, Z.A., and Reimann, J. (1994b). J. Immunol. *152*, 1110–1119.

Schirmbeck, R., Melber, K., Mertens, T., and Reimann, J. (1994c). J. Virol. *68*, 1418–1425.

Schirmbeck, R., Melber, K., Mertens, T., and Reimann, J. (1994d). Eur. J. Immunol. *24*, 1088–1096.

Schirmbeck, R., Böhm, W., Melber, K., and Reimann, J. (1995a). J. Immunol. *155*, 4676–4684.

Schirmbeck, R., Deml, L., Melber, K., Wolf, H., Wagner, R., and Reimann, J. (1995b). Vaccine *13*, 857–865.

Schirmbeck, R., Melber, K., and Reimann, J. (1995c). Eur. J. Immunol. *25*, 1063–1070.

Schirmbeck, R., Böhm, W., and Reimann, J. (1996). Intervirology, in press.

Schuhmacher, T.N.M., Heemeis, M.-T., Neefjes, J.J., Kast, W.M., Melief, C.J.M., and Ploegh, H.L. (1990). Cell *62*, 563–567.

Shirai, M., Pendleton, C.D., Ahlers, J., Takeshita, T., Newman, M., and Berzofsky, J.A. (1994). J. Immunol. *152*, 549–558.

Smith, J.D., Lie, W.R., Gorka, J., Myers, N.B., and Hansen, T.H. (1992a). Proc. Natl. Acad. Sci. USA *89*, 7767–7771.

Smith, J.D., Lie, W.R., Gorka, J., Kindle, N.B., Myers, N.B., and Hansen, T.H. (1992b). J. Exp. Med. *175*, 191–202.

Smith, J.D., Myers, N.B., Gorka, J., and Hansen, T.H. (1993). J. Exp. Med. *178*, 2035–2046.

Srivastava, P.K. (1993). Adv. Cancer Res. *62*, 153–177.

Srivastava, P.K., Udono, H., Blachere, N.E., and Li, Z. (1994). Immunogenetics *39*, 93–98.

Staerz, U.D., Karasuyama, H., and Garner, A.M. (1987). Nature *329*, 449–451.

Stahl, P.D. (1992). Curr. Opin. Immunol. *4*, 49–52.

Steinman, R.M. (1991). Annu. Rev. Immunol. *9*, 271–296.

Steinman, R.M., and Swanson, J. (1995). J. Exp. Med. *182*, 283–288.

Stover, C.K., de la Cruz, V.F., Fuerst, T.R., Burlein, J.E., Benson, L.A., Bennett, L.T., Bansal, G.P., Young, J.F., Lee, M.H., Hatfull, G.F., Snapper, S.B., Barletta, R.G., Jacobs, W.R., and Bloom, B.R. (1991). Nature *351*, 456–460.

Stover, C.K., Bansal, G.P., Burlein, J.E., Palaszynski, S.R., Young, J.F., Koenig, S., Young, D.B., Sadziene, A., and Barbour, A.G. (1993). J. Exp. Med. *178*, 197–209.

Stryhn, A., Pedersen, L.O., Romme, T., Olsen, C.C., Nissen, M.H., Thorpe, J.C., and Buus, S.J. (1996). J. Immunol. *156*, 4191–4197.

Sugita, M., and Brenner, M.B. (1995). J. Biol. Chem. *3*, 1443–1448.

Suss, G., and Pink, J.R. (1992). J. Immunol. *149*, 1334–1339.

Szalay, G., Hess, J., and Kaufmann, S.H. (1994). Eur. J. Immunol. *24*, 1471–1477.

Takahashi, H., Takeshita, T., Morein, B., Putney, S., Germain, R.N., and Berzofsky, J.A. (1990). Nature *344*, 873–875.

Tevethia, S.S., Flyer, D.C., and Tjian, R. (1980). Virology *107*, 13–23.

Townsend, A.R.M., and Bodmer, H.C. (1989). Annu. Rev. Immunol. *7*, 601–624.

Udono, H., and Srivastava, P.K. (1993). J. Exp. Med. *178*, 1391–1396.

Udono, H., and Srivastava, P.K. (1994). J. Immunol. *152*, 5398–5403.

Udono, H., Levey, D.L., and Srivastava, P.K. (1994). Proc. Natl. Acad. Sci. USA *91*, 3077–3081.

Ulmer, J.B., Donelly, J.J., and Liu, M.A. (1994). Eur. J. Immunol. *24*, 1590–1596.

van Binnendijk, R.S., Van Baalen, C.A., Poelen, M.C.M., de Vries, R.R.P., Boes, J., Cerundolo, V., Osterhaus, A.D.M.E., and Uytdehaag, F.G.C.M. (1992). J. Exp. Med. *176*, 119–128.

van Kaer, L., Ashton-Rickardt, P.G., Ploegh, H.L., and Tonegawa, S. (1992). Cell *71*, 1205–1214.

Vega, M.A., and Strominger, J.L. (1989). Proc. Natl. Acad. Sci. USA *86*, 2688–2692.

Vitiello, A., Potter, T.A., and Sherman, L.A. (1990). Science *250*, 1423–1426.

Vitiello, A., Ishioka, G., Grey, H.M., Rose, R., Farness, P., LaFond, R., Yuan, L., Chisari, F.V., Furze, J., Bartholomeuz, R., et al. (1995). J. Clin. Invest. *95*, 341–349.

Weidt, G., Utermöhlen, O., Zerrahn, J., Reimann, J., Deppert, W., and Lehmann-Grube, F. (1994). J. Immunol. *153*, 2554–2561.

Wolf, P.R., and Cook, R.G. (1995). J. Exp. Med. *181*, 657–668.

Wraith, D.C., and Vessey, A.E. (1986). Immunology *59*, 173–180.

Wraith, D.C., Vessey, A.E., and Askonas, B.A. (1987). J. Gen. Virol. *68*, 433–440.

Wu, J.Y., Gardner, B.H., Murphy, C.I., Seals, J.R., Kensil, C.R., Recchia, J., Beltz, G.A., Newman, G.W., and Newman, M.J. (1992). J. Immunol. *148*, 1519–1525.

Wu, J.Y., Gardner, B.H., Kushner, N.N., Pozzi, L.A., Kensil, C.R., Cloutier, P.A., Coughlin, R.T., and Newman, M.J. (1994). Cell Immunol. *154*, 393–406.

Xu, X., and Pierce, S.K. (1995). J. Immunol. *155*, 1652–1654.

Yamada, A., Ziese, M.R., Young, J.F., Yamada, Y., and Ennis, F.A. (1985). J. Exp. Med. *162*, 663–674.

Yasutomi, Y., Koenig, S., Haun, S.S., Stover, C.K., Jackson, R.K., Conard, P., Conley, A.J., Emini, E.A., Fuerst, T.R., and Letvin, N.L. (1993a). J. Immunol. *150*, 3101–3107.

Yasutomi, Y., Palker, T.J., Gardner, M.B., Haynes, B.F., and Letvin, N.L. (1993b). J. Immunol. *151*, 5096–5105.

Yewdell, J.W., and Bennink, J.R. (1992). Adv. Immunol. *52*, 1–123.

Young, J.W., and Steinman, R.M. (1990). J. Exp. Med. *171*, 1315–1332.

Zhou, F., Rouse, B.T., and Huang, L. (1992a). J. Immunol. *149*, 1599–1604.

Zhou, F., Rouse, B.T., and Huang, L. (1992b). Cancer Res. *52*, 6287–6291.

Zhou, X., Berg, L., Abdel-Motal, U.M., and Jondal, M. (1992). J. Immunol. Meth. *153*, 193–200.

Zhou, X., Liu, T., Franksson, L., Ljunggren, H.-G., and Jondal, M. (1995). Scand. J. Immunol. *42*, 66–75.